PROCESS FOR EXTRACTION OF ANTIOXIDANTS FROM SESAME SEED/CAKE

This invention relates to a novel process for the extraction of antioxidant substances from sesame seed/cake. The antioxidant concentrate is prepared from commercially available sesame seed / oilcake (starting material).The antioxidant extract is prepared through selective extraction steps employing organic and or aqueous solvents. The final concentrate obtained is dissolved in a suitable permitted, non-ionic lipid dispersible carrier. The antioxidant so obtained is effective in protecting common vegetable oils / emulsions / lipid systems in foods, cosmetics, pharmaceuticals and the like against oxidative changes at very low concentrations, without imparting color, odor or precipitates. The antioxidant extract /concentrate according to this invention is a mixture of complex lignan compounds like sesamol, sesamin, episesamin, sesamolin, related derivatives, tocopherols, polyphenols/ ferulic acid, denatured proteins, sugars, lipids, minerals and browning products(maillard reaction products) etc. The process described herein minimises interfering compounds without losing much of the antioxidant lignans, by carefully adopting conditions of the different extraction steps. The antioxidant extract from sesame prepared by this novel process can be used as a substitute for synthetic antioxidants such as BHA, BHT or TBHQ that are generally used in vegetable oils, foods, cosmetic and pharmaceutical preparations and is non obvious, as the natural antioxidant extract prepared, is soluble/ dispersible in oil and oil-water emulsion systems.

The process thus aims at generating basic and applied knowledge. The sesame seed or its cake with antioxidant activity can be utilised as a source for natural antioxidant extract, as a substitute for synthetic antioxidants like BHA, BHT and TBHQ. that are banned due to their demerits in many countries. [(Shahidi, F., Natural Antioxidants- Chemistry, Health Effects and Applications, pp. v, AOCS Press (1997)].

The food that we consume contains mainly biomolecules, which are susceptible to attack, by free radicals. The oxidation of lipids by the free-radical chain reaction viz. lipid peroxidation, is a major concern for both consumers and food manufacturers. In order to prevent these unwelcome changes in lipids and foods, modified atmosphere packaging, vacuum packaging, nitrogen gas substitution etc. are practised. The discovery and use of antioxidants to increase the storage life of foods has made possible the marketing of mainly new products and is of direct economic benefit to consumers. Today antioxidants are widely used in processed foods and also in pharmaceuticals, cosmetics, essential oils and plastics for food packaging. The commonly used antioxidants in foods to control oxidation and prevent off-flavour development include Butylated Hydroxy Anisole(BHA), Butylated Hydroxy Toluene(BHT), Propyl Gallate(PG) and tert-Butyl Hydroquinone (TBHQ) at a maximum permitted usage level of 200 ppm.The widespread use of these synthetic compounds is also backed by development of chemical industries. However, their use in foods has been alerted by many agencies due to safety considerations. The possible toxicity of the synthetic chemicals used as antioxidants has been studied for many years (Johnson and Hewgill, 1961; Branen, 1975) as has been cited in the US patent 5,043,100 in1991, by Chang etal. In the same patent cited above, it has been quoted that FDA (Food and Drug Adulteration Act) has expressed concern over the use of BHT as has been reported (Food Chemical News, 1976). The concern stems from scientific literature reviews conducted for the FDA which focused on enzyme inducing effects of BHT on liver and on extraheptic organs, such as lungs and gastrointestinal tract mucosa. As cited in Chang's patent(1991), FDA had expressed concern over the effect of BHT on the conversion of other ingested materials into toxic or carcinogenic substances by the increase of microsomal enzymes. As a result because restrictions have been placed upon the use of such synthetic antioxidants by many European and Asian countries. As cited in the same patent by Chang et al (US Patent No. 5,043,100 August 27,1991) ; BHA has been removed from the GRAS list by the FDA after Nobuyuki Ito of Nagoya City

University Medical school in Japan,(1982), reported findings showing BHA to be carcinogenic in rats, BHA is in the process of being carefully scrutinized. TBHQ has not been approved in Japan, Canada and certain European countries. These countries hold that there is insufficient information available on safety of the antioxidant. Moreover, the high cost of synthetic antioxidants in addition to the dubious safety aspects warrants the investigation, preparation and evaluation of natural antioxidants suitable for food and vegetable oil protection. Added to this, there is a tendency for the consumers to reject foods containing synthetic additives and consequently it is a great marketing advantage for the manufacturer to claim a food product as 'all natural'. Efforts have already been started worldwide to find alternates to check lipid oxidation and rancidity development in foods especially naturally occurring components from edible sources. The major dietary antioxidants identified so far are tocopherol, carotenoids, ascorbate etc.

Since an antioxidant is an unavoidable additive, very soon the food, pharmaceutical and cosmetic industries will have no practical options because the only common natural antioxidant viz. mixed tocopherols has only weak antioxidant properties. The use of carotenoids is limited due to its high lipid solubility and intense coloration even at low ppm levels of 50 -100 ppm. Therefore, the need for safe and effective natural antioxidants is urgent and genuine.

The prior art on natural antioxidants for food uses have patents related to the antioxidants from tea (Chang etal,5,043,100 ,1991,US Patent),(United States Patent on Lipid soluble green tea catechin antioxidant solutions, No.5,527,552, 1996 by Todd, Jr. and Paul H.) and from rosemary and other herbs(US Patent 5,753,697 1998 by Joyeux etal on 'Method and pharmaceutical compositions containing rosmanol derivatives'.),(US Patent 5,017,397,1991 by Nguyen etal on 'Process for extracting antioxidants from Labiatae herbs'.). The rosemary extracts at 0.02% level is claimed to show antioxidant activity at least equal to synthetic antioxidant mixture of BHA/BHT(1:1).The disadvantage of these natural antioxidant extracts is that they can not be used at higher concentrations, especially the odoriferous ones, since they impart undesirable flavor taints to

foods and other products. In the case of phenolic extracts, the solubility of such antioxidants in vegetable oils is disputable since they are lipophobic. Though they are compounds with confirmed antioxidant activity, extracts containing phenolic/flavonoids may not be suitable for oils and fats due to their poor dispersibility in pure lipids.

At this juncture the protection of foods and oils against oxidation becomes inevitable since free radicals produced during the autoxidation of lipids are injurious. The major recommendation by scientific community to industry is to utilise natural antioxidants available from different sources. At present,Vitamin E, Vitamin C, flavonoids etc are reported as effective natural antioxidants capable of protecting oils from autoxidative changes (N.Nakatani In : *Natural Antioxidants. Chemistry, Health Effects and Applications*. Pp 64-75. Fareidoon Shahidi (Editor), AOCS Press(1997).], [N. Ramaratnam, H. Ochi, and M. Takeuchi. In: *Natural Antioxidants. Chemistry,Health Effects and Applications*, pp 76-95. Fareidoon Shahidi (Editor), AOCS Press(1997)]. However, solubility of the natural antioxidant in oil phase is important if they have to be used with vegetable oils commercially produced. Thus Vitamin C and flavanoids will have to be made miscible in oil phase if they are used with vegetable oils as against aqueous system with oil/water emulsion. This is possible only through modification of their structure through derivatisation by chemical/enzymatic methods. Alternately, effective oil soluble antioxidants and their extracts have to be developed for vegetable oil protection.

Sesame oil which is highly unsaturated (IV 104-109) and commercially extracted by established milling processes is well known for its unusual stability. This is attributable to the antioxidant components and lignans like sesamol (structure 1), Sesamin (structure 2),and sesamolin (structure 3); sesamol is reported to be produced from sesamin during refining/frying steps [SeeY.H.Hui(Editor)*Bailey's Book on Industrial Oils and Fat Products*.5th Edn.,Vol.2.,Pp 457-495, Wiley International Publication (1996)].In the present invention, a process for extracting antioxidants from seeds/cake is described and the product, namely the antioxidant extract/concentrate is tested by different

methods to ascertain its activity, in protecting crude or refined vegetable oils like soyabean, sunflower,safflower, groundnut oil etc.

Sesamol, the antioxidant compound reported to be present in traces in sesame oil is produced synthetically and added as antioxidant in fat/food products [See K.Kikugawa etal.,*J.Amer.Oil Chem.Soc.*,**60**,1528-1533(1983)as cited in*Bailey's Book on Industrial Oils and Fat Products*.5th Edn.,Vol.**2**.,Wiley International Publication(1996);Hiromi Yashida etal.,*J.Sci.Food Agric.*,**79**,220 (1999)]. Sesamol is reported to be present in sesame oil extracts from sesame in trace quantities. In the present invention, sesamol, in addition to other lignans, is extracted from sesame seed/cake into an effective antioxidant extract. Sesamol being polar is not extracted into hydrocarbon solvents; it is extracted into oil only in traces in the conventional milling process.

Investigations conducted on the unusual stability of sesame oil inspite of its unsaturation has led to the finding that compounds other than tocopherol are naturally protecting the oil from autoxidative changes. Prior investigation by Indian [Satchidanandan Subramanian etal., *J.Nutr.*,**123**(11) 1852-1858 (1993)] as well as Japanese workers[Fukuda etal., *J. Amer. Oil Chem. Soc.*, **63**(8) 1027-1031(1986)] have shown the presence of compounds like sesamol, sesamolin, sesamin, sesaminol etc. in the analysis of hexane extracts of sesame seed using HPLC;sesamin, sesamolin have been reported in greater quantitites and sesaminol and sesamol only in traces. Mimura etal (US Patent 5,132,294;July 21,1992) have patented the process for preparing antioxidative glycosides and the composition containing the same. In this invention, the applicants have prepared the glycosides from plant cell cultures derived from sesame (Sesamum indicum L.) which shows antioxidant activity against linoleic acid oxidation as assayed by the thiocyanate method and with (rabbit) erythrocyte ghost cell methods. Aqueous ethanolic/acetone extracts of sesame seeds have also been reported to contain glycosides of lignans having antioxidant activity. [Katsuzaki etal., *Phytochemistry*, **35**(3), 773-776 (1994)]. The glycosides are reported to have antioxidant activity in cell culture studies. The use of glycosides in cosmetic preparations is reported in prior art. However, the glycosides, derived naturally or

artificially from sesame, are not dispersible in oil systems for protection and are suitable for emulsions only. Also another US patent has been registered by Kawakishi etal in 1997 (US Patent 5,606,035) in which extracts or compositions from sesame and other added antioxidants such as tocopherols , phenolics etc.also, are claimed to have hypocholestemic effect in animals. This is a patented pharmaceutical formulation.

Following are the main points of difference of our process and prior art information.

This also explains how our work is different form others', in what all ways ours is non- obvious and how it is innovative.

Highlights of prior art information	**Non-obviousness/improvement of our process, compared to already existing knowledge.**
Method of extraction:- Most of the earlier reports had analysed sesame oil obtained commercially or by hexane extraction of the seeds for the analysis of lignans and antioxidant compounds by HPLC (Please see reference paper attached as Annexure IV and copies of reference papers viz.(i.) review on 'The Chemistry and Physiological Functions of Sesame' by Mitsuo Namiki and ii) Excerpts from Bailey's Industrial Oil &Fat Products, Vol.II , Chapter on 'Sesame oil' that was sent along with the original text.). In a recent report by Su Noh Ryu etal, seeds have been reportedly extracted with solvents like acetone and 80% aqueous alcohol (80 alcohol: 20 water, vol/vol.) ,treated with enzymes and analysed for antioxidant compounds such as	We had systematically extracted sesame seed/cake with different solvents .The details of cake extracts are given in Table 3, attached as Annexure VI . The % of extract and the composition of it with respect to antioxidant compounds was estimated by HPLC. Results showed that the crude extracts contained sesamol and the lignans in the range of 0.8-1.0 % only. The purified extract prepared by our process steps consisting of , for example defatting, water - washing and methanol extraction resulted in an antioxidant concentrate with 14.0% lignans such as sesamol, sesamin and sesamolin. (In addition to above three compounds, other constituents such as sesaminol, sesamolinol and or their glycosides which are shown to be present in

sesamin, sesamolin and glycoside derivatives of sesaminol and sesamolinol(copy of reference paper attached in Annexure IV). There is only insufficient data available on nature and quantity of different antioxidant compounds.	our extract by HPLC profile, are excluded for calculation of the total lignan content. Still, there is improvement in content of antioxidant compunds.) There is a significant improvement of (nearly 10 times on extract weight basis)antioxidant content by our process. The radical scavenging activity of the latter extract as measured by DPPH method, increased 24 times as a result of our process. This is clear from the Tables attached as Annexure VI. (These were referred as Tables 1 and 2 in the original of the text). The protection of soybean oil for example, was also better for the purified extract compared to crude extract as can be seen from Figures 1 and 1a included in the Annexure VII.
All the antioxidant compounds are not optimally extracted in the single step extraction procedures implicitly or explicitly described in the prior art.	In our process, stepwise extraction is followed and conditions are so chosen as to selectively enrich antioxidant compounds and the process is an improvement of existing knowledge.The process is also non -obvious.
The US patents (no.4,427,694 and no.5,637,610) on biological effects of some specified compositions consisting of sesame lignans protects the biological effects of the compositions. In addition to sesame lignans, they contain added antioxidants such as tocopherol.(Copies of first pages of these Patents were already sent with Text earlier.)	Our extract is purely from sesame seed /cake and does not contain additional antioxidants. Instead, we are concentrating the antioxidant content through limited steps that can be practically followed by industry.
Commercially available antioxidant extracts such as rosemary are used at 0.02, 0.05 and 0.1% levels as per reports.(Please see the paper on Antioxidant Activity of Rosemary Extracts	Our extract prepared according to the patentable process, need to be used at much lower concentrations of less than 0.01%(ie.100 ppm) and is dispersible in oil and water- oil

included in Annexure IV).	emulsions.
Cost of the currently used synthetic antioxidants and commercially available natural antioxidant have been compared with the sesame antioxidant usage.	Sesame antioxidant will be economical.

Summary of the Invention

Though sesame antioxidants are reported in prior art, there is no disclosure of a process for an antioxidant extract from sesame seed or cake that is an effective substitute for synthetic antioxidants for oil/food protection .Thus the object of this invention is to deliver a natural antioxidant extract from an edible source like sesame seed or sesame cake which is a byproduct of the oil industries, through simple extraction steps. The antioxidant extract according to this invention consists of mainly antioxidant lignan compounds like sesamol, sesamin, episesamin sesamolin, glycosides and related compounds, phenolic acids, denatured proteins, lipids, soluble sugars, minerals etc. The extraction of antioxidants has been tried with different organic solvents of hydrocarbon, alcohol, alkyl ketone and ester types. The separation, idetification and quantification of peaks was carried out by HPLC analysis. The antioxidant activity was evaluated by potassium ferricyanide method[See G.C.Yen and P.D.Duh.,*J.Amer.Oil Chem.Soc.*,**70**,353-386(1993)].,ferric thiocyanate method [See G.C.Yen and C.L.Hsieh.,*J.Agric.Food Chem.*,**46**,3952-3957(1998)] as biochemical methods. The antioxidant efficiency was also tested by evaluating the oxidative stability of refined soybean oil in heated conditions, using Differential Scanning Calorimeter (DSC) and also by invitro stability studies with refined oils by Schaal Oven Test method[Owen R Fennema in: *Principles of Food Science.Part 1.Food Chemistry*,Pp166-168, Marcel&Dekker Inc.(1976)] which are described subsequently. In the present invention, the natural antioxidant concentrate from an edible source like sesame seed / cake is prepared by organic and or aqueous extractions .The extract(s) so obtained is capable of protecting

commonly used vegetable oils, foods etc. against autoxidation and exhibits high level of radical quenching activity as explained later.

Thus , it is an object of this invention that sesame antioxidants can be extracted by alcohols, ketones, esters or substituted hydrocarbon solvents, for 10 hrs to 7 days . The extraction can be carried out over a temperature range of 25-85°C. The extract can be concentrated under reduced pressure by usual laboratory practices and dissolved in a non-ionic, non- aqueous permitted organic solvent or food carrier.

It is also another object of this invention that the starting material can be first treated with hydrocarbon solvents such as pentane, hexane, heptane or mixtures there of, for defatting over a temperature range of 25-85 ° C for 10-24 hours, to facilitate subsequent antioxidant extraction.

It is also another object of this invention that the sesame seed/cake is extracted with organic solvents belonging to the group comprising of methanol, ethanol, isopropanol, acetone, dichloromethane ,ethyl acetate, etc. over a temperature range of 25 to 85°C and removal of extraction solvent by usual laboratory practices,under reduced pressure (100 -150 mm) leads to an antioxidant extract containing 5.0 to 20.0% of the lignans on dry weight basis of the extract, according to HPLC analysis..

In another object of this invention, the above extract containing mainly lignan compounds like sesamol, sesamin, episesamin, sesamolin, glycosides and other derivatives including lignan dimers, lipids, soluble sugars, proteins, minerals, browning products etc. is capable of protecting commonly used vegetable oils like soybean oil, sunflower oil, safflower oil and ground nut oil at concentrations equal to or lower than those of currently used synthetic antioxidants like BHT and TBHQ.

In another object of this invention, the sesame seed/cake (after extraction with hydrocarbon solvents), is optionally washed with water, with or without salts such as sodium chloride at 3-10 % (w/v) levels, to remove substances such as

carbohydrates and or proteins interfering with dispersibilty/ antioxidant activity of the extract.

It is also yet another object of this invention that the starting material, if subjected to above step, is dried below 70^0C .

Still another object of this invention is to provide a natural antioxidant extract for protection of vegetable oils, that contains natural component(s)such as sesamol, sesamin, episesamin, sesamolin etc, which are reported to have beneficial biological effects such as hypocholestemic, anti-hypertensive effect, anti-aging effect, anticancer effect etc.[as cited in the review 'The chemistry and Physiological Functions of Sesame'. Mitsuo Namiki. *Food Reviews International*,11(2),281-329(1995)].

Still another object of this invention is to provide an antioxidant extract from an edible/natural source which is also shown to have beneficial health effects like antioxidant /anticancer effects.

It is also another object of this invention that the lipids stabilised with sesame extracts according to this invention have positive effects on human health and can be considered as superior to the same lipid stabilised with common synthetic antioxidants.

Still another object of this invention is that the synergy of various lignan and non lignan compounds present in this extract is effective in protection of vegetable oils/foods, at lower concentrations is better than single antioxidants such as sesamol .

It is also yet another object of this invention to provide a natural antioxidant extract that is oil dispersible and does not impart any colour, aroma or flavour when used at lower or moderate (permissible) levels of concentrations.

It is still another object of this invention to provide an antioxidant extract that is efficient in protecting vegetable oils at lower concentrations of 0.005% to 0.02% which is less than that of other natural antioxidants under commercial use and also synthetic antioxidant (0.02%).

It is also another object of this invention that the antioxidant extract when used at 50 to 200 ppm levels contains the actual antioxidant compounds, namely the lignans at 1 to 30 ppm levels only, which is extremely low compared to currently used levels of BHT or TBHQ and other reported natural extracts.

According to another object of this invention, one of the identified starting materials ,namely sesame cake, a byproduct of the sesame oil industry currently used mainly as a cattle feed , is value-added by this process resulting in better by-product utilisation.

According to yet another aspect of this invention the final residue obtained after different steps of extraction(s) of the cake is still high in protein content and may be utilised as cattlefeed or for similar preparations.

The process also uses sesame seeds as the starting material and after the stages of hydrocarbon solvent extraction, oil can be recovered from sovent by usual methods of solvent removal and the final residue can be utilised in the same way as with cake as the starting material.

The antioxidant extract can be used to protect foods, cosmetics, pharmaceuticals etc. also which are currently protected by synthetic antioxidants.

The process can also be carried out with sesame seeds of Red(brown),Black, White and wild varieties(eg.Sesamum malabaricum).

Additional objects and advantages will be apparent from a consideration of the following description and in part will become apparent to those skilled in the art upon examination of the following or may be learned by practice of the invention and may be realized and attained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

In order to achieve the foregoing and other objects mentioned and the present invention as embodied and broadly described herein, the process of this invention of extracting antioxidants from sesame seed/cake comprises extracting the starting material with hydrocarbon solvents such as pentane, hexane, heptane or mixtures thereof at 25-85° C preferably at 25-60°C at 1:1 to 1:7 ratio for 10-24

hrs, followed by the optional step of washing with water at 1:1 to 1:5 ratio preferably at 1:2 to1:5 ratio 3 to 5 times or with 3- 10 % NaCl 1-3 times, followed by water washing 1-4 times and drying below 70 °C under a draft of air including sun drying, followed by extraction with organic solvents such as alcohols/ esters/ketones having C1- C5 carbons at a ratio of 1:1 -1:7, using usual laboratory extraction procedures such as soxhlet extraction, refluxing, stirring, percolation etc. for 10 hrs to 7 days(when temperatures < 30°C is employed), to extract substantially the antioxidant compounds. The extracts after filtration through a simpler filter aid can be concentrated under reduced pressure at temperatures below 60 ° C in a rotary evaporator and the residue can be redissolved in ethanol or any permitted additive carrier such as ethylene glycol, propylene glycol etc.

The extract is concentrated by removing the solvent under reduced pressure towards end and redissolved in pure ethanol or permitted carriers The residual sesame meal after this final extraction can be reused as cattlefeed, since it is still rich in proteins (37-50 %), fibre(8-10 %) and minerals (11-15 %).

The present invention also relates to a process of producing a natural antioxidant concentrate containing 5 to 20 % lignans, as analysed by HPLC.

A typical sample of sesame seed has 40-50% fat content and cake has approximately 6 -8 % :moisture levels are less than 10 % in both cases.The starting material is powdered with a steel mortar and pestle or with a similar arrangement and the material is defatted fully or partially with hydrocarbon solvents such as hexane or petroleum ether in the ratio of 1:2 to 1:5 preferably 1:2: 1:3. The usual laboratory set up like hot extraction by soxhlet, refluxing, cold extraction by stirring etc. are employed.

Accordingly 10 g. to 100 g samples can be extracted with solvents such as pentane, hexane, heptane or mixtures thereof over a temperature range of 25 -85 o C for 10 to 24 hours for defatting. The defatted cake meal can be extracted with alcoholic solvents belonging to a group of solvents comprising of methanol, ethanol, isopropanol etc. or ketones like acetone or esters like ethyl acetate by the usual laboratory procedures of extraction by refluxing, soxhlet, stirring or elution

through a column packed with the substance. The extraction time may range from 10 hours to 7 days as mentioned earlier.

The antioxidant efficiency of the extract was evaluated by studying the oxidative stability of commonly used RBD vegetable oils such as soyabean,safflower,sunflower,ground nut oil

The oxidative efficiency of the extract was evaluated by studying the oxidative stabilty of commonly used RBD vegetable oils such as soyabean, safflower, sunflower, ground nut oils(without any added synthetic antioxidants) during storage at ambient(28$\pm$ 2°C) and higher temperatures (60 °C)according to the Schaal Oven Test method. To carry out test, about 15-100g oil samples were taken in uniform containers, in duplicate with or without headspace and loosely stoppered/covered and incubated at 60 ° C. In an actual embodiment of the process, the stability studies were conducted using control and experimental samples of the natural antioxidants ranging from 3 to 1000 ppm preferably in the range of 5 - 200 ppm were used in the experiment. Permitted antioxidants like BHT and TBHQ were also studied over a concentration range of 50-200 ppm. During storage at 60 °C, the oil samples were analysed periodically for the peroxide value(AOCS method), Diene value(IUPAC method) and secondary oxidation changes by the anisidine value according to the Jirusova etal.,*Nahrung*.,**19**,319(1975) and fatty acids by GC analysis at regular intervals.

The levels of use of the sesame antioxidant extracts required were much lower(50 to <200 ppm) than the synthetic counterparts which were used at 200 ppm level. The protection offered by sesame extract was comparable/better with that of BHT at 200 ppm level, in both ambient and incubated storage studies. The results are given in Figs.1,2,3,4,5.

The radical quenching efficiency of the extract was also tested by widely reported standard methods like di-phenyl picryl hydrazyl (DPPH) radical according to the methods of W.Brand Williams, M.E.Cuvelier and C. Berset.,*Lebesm.-Wiss. U-Technol.*,28,25-30(1995) and. the efficiency established as shown in Tables 1&2.

The oxidative stability of vegetable oils containing synthetic antioxidants (BHT and TBHQ at 50- 200ppm) and sesame extract at different levels 3 to 1000 ppm were also tested in the Differential Scanning Calorimeter (Mettler Toledo, model 821) under flow of oxygen (40 ml/min.). The heating regime followed was isothermal at 150 ° C and from the curves obtained (Figs. 6,7), the 'onset-time' of oxidative changes under identical conditions could be found out by making use of an inbuilt program of the instrument, the induction period represented by the onset time, can be correlated with the onset of rancidity. The experiments showed increased oxidative stability for vegetable oils containing the extract.

Detailed Legend for Figures

Fig.1 Peroxide Value (milli equivalents of oxygen / Kg.) of Soybean Oil stored at 60 ° C

Control : This represents soybean oil without any added antioxidants.

TBHQ 200 ppm: This represents the sample of soybean oil containing the synthetic antioxidant viz. TBHQ at the level of 200 ppm ie.200mg per Kg. of the oil.

BHT 200 ppm : This represents the sample of soybean oil containing the synthetic antioxidant BHT at the level of 200 ppm ie.200mg per Kg. of oil.

Sesame 5 ppm : This represents the sample of soybean oil containing sesame antioxidant extract at the level of 5 ppm ie.5 mg per Kg. of oil.

Sesame 10ppm : This represents the sample of soybean oil containing sesame antioxidant extract at the level of 10 ppm ie.10 mg per Kg. of oil.

Sesame 50ppm : This represents the sample of soybean oil containing sesame antioxidant extract at the level of 50 ppm ie.50 mg per Kg. of oil

Sesame100ppm : This represents soybean oil containing sesame antioxidant extract at the level of 100 ppm ie. 100 mg per Kg.

From the plot, it can be seen that sesame extracts, even at low concentrations are more efficient than BHT at maximum permitted levels.

Compare figure 1 and figure 1a for improved antioxident efficiency of purified sesame cake extract over crude extract.

Fig.2 Peroxide Value (milli equivalents of oxygen/ Kg.) of Safflower Oil stored at 60 °C

Control : This represents safflower oil without any added antioxidants.

TBHQ 200 ppm: This represents the sample of safflower oil containing the synthetic antioxidant viz. TBHQ at the level of 200 ppm ie.200mg per Kg.of the oil.

BHT 200 ppm : This represents the sample of safflower oil containing the synthetic antioxidant BHT at the level of 200 ppm ie.200mg per Kg. of oil.

Sesame 5 ppm : This represents the sample of safflower oil containing sesame antioxidant extract at the level of 5 ppm ie.5 mg per Kg. of oil.

Sesame 10ppm : This represents the sample of safflower oil containing sesame antioxidant extract at the level of 10 ppm ie.10 mg per Kg. of oil.

Sesame 50ppm : This represents the sample of safflower oil containing sesame antioxidant extract at the level of 50 ppm ie.50 mg per Kg. of oil

Sesame100ppm : This represents safflower oil containing sesame antioxidant extract at the level of 100 ppm ie. 100 mg per Kg.

From the plot, it could be seen that sesame extracts, at different levels were equally efficient than BHT at maximum permitted levels.

Fig.3 Peroxide Value (milli equivalents of oxygen/ Kg.) of sunflower oil stored at 60 °C

Control : This represents sunflower oil without any added antioxidants.

TBHQ 200 ppm: This represents the sample of sunflower oil containing the synthetic antioxidant viz. TBHQ at the level of 200 ppm ie.200mg per Kg.of the oil.

BHT 200 ppm : This represents the sample of sunflower oil containing the synthetic antioxidant BHT at the level of 200 ppm ie.200mg per Kg. of oil.

Sesame 5 ppm : This represents the sample of sunflower oil containing sesame antioxidant extract at the level of 5 ppm ie.5 mg per Kg. of oil.

Sesame 10ppm : This represents the sample of sunflower oil containing sesame antioxidant extract at the level of 10 ppm ie.10 mg per Kg. of oil.

Sesame 50ppm : This represents the sample of sunflower oil containing sesame antioxidant extract at the level of 50 ppm ie.50 mg per Kg. of oil

Sesame100ppm : This represents sunflower oil containing sesame antioxidant extract at the level of 100ppm ie. 100 mg per Kg.

From the plot, it can be seen that sesame extracts, even at low concentrations are more efficient than BHT at maximum permitted levels.

Fig.4. Diene Value of Safflower oil stored at 60 ^{o} C.

Control : This represents safflower oil without any added antioxidants.

TBHQ 200 ppm: This represents the sample of safflower oil containing the synthetic antioxidant viz. TBHQ at the level of 200 ppm ie.200mg per Kg.of the oil.

BHT 200 ppm : This represents the sample of safflower oil containing the synthetic antioxidant BHT at the level of 200 ppm ie.200mg per Kg. of oil.

Sesame 5 ppm : This represents the sample of safflower oil containing sesame antioxidant extract at the level of 5 ppm ie.5 mg per Kg. of oil.

Sesame 10ppm : This represents the sample of safflower oil containing sesame antioxidant extract at the level of 10 ppm ie.10 mg per Kg. of oil.

Sesame 50ppm : This represents the sample of safflower oil containing sesame antioxidant extract at the level of 50 ppm ie.50 mg per Kg. of oil

Sesame100ppm : This represents safflower oil containing sesame antioxidant extract at the level of 100 ppm ie. 100 mg per Kg.

From the plot, it could be seen that sesame extracts, at different levels were equally efficient compared to BHT at maximum permitted levels.

Fig.5. Diene Value of Sunflower oil stored at 60 ° C.

Control : This represents sunflower oil without any added antioxidants.

TBHQ 200 ppm: This represents the sample of sunflower oil containing the synthetic antioxidant viz. TBHQ at the level of 200 ppm ie.200 mg TBHQ per Kg.of the oil.

BHT 200 ppm : This represents the sample of sunflower oil containing the synthetic antioxidant BHT at the level of 200 ppm ie.200mg BHT per Kg. of oil.

Sesame 5 ppm : This represents the sample of sunflower oil containing sesame antioxidant extract at the level of 5 ppm ie.5 mg sesame extract per Kg. of oil.

Sesame 10ppm : This represents the sample of sunflower oil containing sesame antioxidant extract at the level of 10 ppm ie.10 mg extract per Kg. of oil.

Sesame 50ppm : This represents the sample of sunflower oil containing sesame antioxidant extract at the level of 50 ppm ie.50 mg extract per Kg. of oil

Sesame100ppm : This represents sunflower oil containing sesame antioxidant extract at the level of 100 ppm ie. 100 mg extract per Kg.of oil.

From the plot, it could be seen that sesame extracts, at lower levels were more efficient compared to BHT at maximum permitted level of 200 ppm..

Fig.6. DSC profile of oxidative stability of soybean oil containing synthetic and sesame antioxidants at different concentrations.(ppm)

DSC experiments: Differential Scanning Calorimeter . The Mettler Toledo instrument used for carrying out the studies, is configured to follow oxidative stability of vegetable oils at a desired higher temperature under oxygen flow. Vegetable oils were subjected to temperature programmed heating regime under flow of oxygen and it was found that 150 $^{\circ}$C was the temperature of inflexion for most of the vegetable oils. Hence, for further studies, isothermal heating of oils at 150 ^{0}C, under a stream of oxygen at 40 ml per min was selected to evaluate the oxidative stability. With the help of an inbuilt program of the instrument, the induction period in 'time- units' could be calculated. This is indicated in the diagram as 'onset ' time. Longer 'onset ' time indicates better protection. Crude and Purified extracts were subjected to study. DSC profiles of purified sesame extracts have been included here.

In Fig.6, the oxidative stability of soybean oil stored at 60 $^{\circ}$C for 7 days has been depicted.(The storage study continued for one month. The extent of protection as measured in DSC was studied here.). Each experiment such as Control alone, (soybean oil + BHT), (oil + TBHQ), (oil + Sesame extract at 10 ppm, 50 ppm, 200 ppm) etc were carried out separately .For comparison sake, the different profiles have been overlaid in the same figure. In Fig.6, the concentrations of BHT and TBHQ are 200 mg per Kg.of oil. For comparison, the profile of oil containing sesame extract at 50 ppm is also

depicted in the overlaid diagram. It could be seen that sesame extract at 50 ppm is equally efficient as BHT at 200 ppm.

Explanation of other terms in the Figure.

1.The DSC profile marked as '1' represents soybean oil control sample stored for one(1) week Onset time 6.27 minutes.

2.The DSC profile marked as ' 2 ' represents stored (one week, 60 ^{0} C) soybean oil containing BHT at 200 mg per Kg of oil concentration. Onset time 7.30 minutes.

3.The DSC profile marked as ' 3 ' represents stored (one week, 60 ^{0} C) soybean oil containing TBHQ at 200 mg per Kg. of oil concentration. Onset time 20.48 minutes.

4.The DSC profile marked as ' 4 ' represents stored (one week, 60 ^{0} C) soybean oil containing sesame antioxidant extract at 50 mg per Kg. of oil concentration. Onset time 7.30 minutes , same as BHT at 200 mg per Kg level.

These results support the data on Peroxide Value estimations and the finding that sesame extract at lower concentration is comparable with BHT at maximum levels, in protecting stored vegetable oils from oxidative changes.

Fig.7.DSC profiles of safflower oil containing synthetic and sesame extracts at different concentrations.

As explained elsewhere, the Figure represents overlaid DSC profiles of safflower oil containing synthetic and sesame antioxidants at few select concentrations.
The onset time has been recorded in the printout of the profile.

Fig.7 represents DSC profiles of oxidative stability of fresh samples of safflower and sunflower oils .

1.The profile marked as '1' represents control sample of safflower oil. Onset time 4.72 minutes.

2. The profile marked as '2' represents safflower oil containing sesame extract at 50 mg per Kg of oil concentration. Onset time is 5.80 minutes.

3.The profile marked as ' 3 ' represents safflower oil containing sesame extract at 100 mg per Kg. of oil concentration.Onset time 7.22 minutes.

4.The profile marked as ' 4 ' represents safflower oil containing BHT at 200 mg per Kg of oil concentration. Onset time 7.23 minutes.

5.The profile marked as ' 5 ' represents safflower oil containing TBHQ at 200 mg. Per Kg of oil concentration.Onset time 10.01 minutes.

Results show that in the case of Safflower oil , sesame extract is effective at lower levels and at 100 mg/Kg level offers protection comparable with BHT at 200 mg/ Kg. concentration.

Fig. 8. HPLC Profile of sesame extract

The Indian ink drawing sent along with the patent text as Fig. 8, is the HPLC profile of Sesame cake extract (crude extract of sample).The identified peaks that are confirmed have been marked as 1,2,3 etc. Calibration of the peaks have been carried out with standard sesamol which is a known antioxidant constituent, characteristic of sesame and has matching wavelength of maximum absorption of 296 nm (λ_{max}) and comparable

molar absorption coefficient of ε_{max} 29.7 with other compounds identified and marked. Thus, the absorption characteristics of sesamin, and sesamolin as quoted in Bailey's Industrial Oil and Fat Products.(vol.2),V edn.1996. p 470, are: Sesamin λ_{max} 287 and ε_{max} 23.0 ; Sesamolin λ_{max} 288..5 and ε_{max} 21.8

Fig.9. HPLC profile of sesame extract (attached) is the actual print out from the instrument of the stored data on HPLC profiles.

One photocopy of the above HPLC profile is also enclosed in which the areas of identified and tentatively identified components (glycosides of sesaminol) have been calculated (The lignan content based on calibrated area of the numbered peaks was 7836 ppm (0.78%) on extract weight basis. On raw material weight basis, the value is 1578.9 ppm.

These have been attached for perusal as per the telephonic conversation and suggestion of Head, IPMD cell.

Fig.10. HPLC profile of sesame extract prepared according to the patentable process, given as Fig. 10 , . is the actual print out of the stored data from the instrument on HPLC profiles.

One photocopy of the above HPLC profile(Fig.10) is also enclosed in which the areas of identified and tentatively identified components (eg.glycosides of sesaminol) have been calculated. The lignan content based on calibrated area of the numbered peaks was 1,40,914 ppm (14.09 %) on extract weight basis. On raw material weight basis, the value is 6887 ppm

These have been attached for perusal as per the telephonic conversation and suggestion of Head, IPMD cell.

The present invention is further described in the following examples which are only illustrative but not limitation for the scope of present invention.

Example 1.

100g of the commercial sesame seed was powdered in a steel mortar and pestle and defatted by stirring with 400ml of hexane in a 5 litre beaker with occasional stirring under ambient conditions (30 $\pm$ 2 $^{\circ}$ C) and covered with petridish for four hours. After four hours, the supernatant layer was decanted off and the residue in the container was stirred with 400ml of fresh charge of hexane and stirred for 4 hours, with frequent stirring and supernatant solvent layer was decanted off as mentioned above. The extraction procedure was repeated as described above 4 more times. The residue(45g) was air dried to remove traces of solvent.

Example 2.

The starting material can be defatted by soxhlet extraction also. In a typical experiment, 100g of commercially available sesame cake was powdered in a steel mortar and pestle and enclosed in a Whatman 1 filter paper thimble and kept in a soxhlet extractor of 2 litre flask capacity and 700ml extractor capacity. The soxhlet extraction was continued for 12 hours, in a typical embodiment of the process. The solvent was removed by distillation and traces removed under partial vacuum(<100mm of Hg.).The residue weighed 92 g in a typical experiment..

Example 3

The defatted residue(90 g) of cake was treated with 500ml of sodium chloride solution of 10 % w/v. Soaking time was 1 hour with occasional stirring and decanted. This was repeated at 1:3 ratio two more times. This was followed by washing with water at 1:3 ratio for three(3) times. The residue obtained after these washings was dried below 70^0C in a drier with a draft of air,to get ~63g.

Example 4

The defatted residue(~ 45 g) obtained from seed was washed with 300ml of water. Soaking time was 1 hour with occasional stirring and decanted. This was repeated with 200 ml water, 4 times. The residue obtained after these washings was dried below 70^0C in an oven with a draft of air to get ~26g of meal.

Example 5

The residue obtained after defatting and washing as described in examples (2) and (3) was extracted with methanol in a soxhlet extractor. 10g of the defatted, brine/water washed, residue was extracted in a soxhlet extractor with 200 ml methanol in a soxhlet extractor with 70ml capacity upto siphoning level of the extractor. The extraction continued for 16 hours. The solvent was removed from the extract by distillation and finally in a rotary evaporator and dried at 60^0C for 2 hours. Cooled in desiccator and weighed. The weight was 1.0g. About 80% of this extract could be redissolved in 100ml of ethanol. to have a stock solution of the antioxidant.

Example 6.

HPLC analysis of antioxidant extract by Reverse phase and quantification of separated peaks was performed as follows. In an actual experiment a Shimadzu make LC-10 A D analytical HPLC equipped with a Rheodyne injector with 20µl sample loop,

a UV-Visible detector and C-R7Ae model data analyser was used. The column connected was μ-bondapak column (4.6 mm x 25 cm) and solvent system tried was methanol: water (70:30). The UV-detector was set at 290nm Fig.8 represents the HPLC chromatogram. Quantitation of separated peaks was done by calibrating with standard sesamol(Sigma-Aldrich Co., USA).The peaks were identified from coinjection of standards as well as from reports. On HPLC analysis, the extract from Example 5, showed a total lignan content of 55 mg. on the extract weight basis; the major antioxidant/lignans being sesamol 11 mg, sesamin 39 mg and sesamolin 3 mg respectively.

The comparison of free radical scavenging activity is shown in Table 1:

Table 1.

Sl.No	Sample	Conc. Of Antioxidant	Free radical Scavenging Effect after 30 min.
1	B H T	20 μ M	81.61
2	T B H Q	20 μ M	98.88
3	Catechin	20 μ M	98.51
4	Tannic Acid	20 μ M	98.88
5	Sesame cake extract with MeOH	1.925 mg/ml	98.43

The radical quenching activity of antioxidants is shown in Table 2:

Table 2

Sample	EC_{50}	Antiradical power* (ARP)

Sesamol	75	$13x10^{-3}$
α-tocopherol	200	$5 X 10^{-3}$
Ascorbic acid	125	$8 X 10^{-3}$
BHT	300	$3.3 X 10^{-3}$
TBHQ	60	$16 X 10^{-3}$
Sesame cake extract	$154 X 10^{3}$	$0.648 x 10^{-5}$
Sesame cake extract (purified)	$6.4 X 10^{3}$	$15 x 10^{-5}$
Sesame seed extract	$30 X 10^{3}$	$3.33 X 10^{-5}$

***Antiradical Power(ARP) = 1 / EC_{50}**

As a result of the purification steps,the antiradical power improved nearly 24 times as evident from the values of crude and purified extracts respectively

The results of crude extraction studies of sesame seed/cake * is shown in Table 3:

Table 3.

Sample	Extract weight (%)	Antioxidant lignans in ppm (ie.mg. per Kg.)			
		Sesamol	Sesamin	Sesamolin	Total
1.Sesame cake extracted with Methanol	20.2	2359 477	4431 895	936 189	8283 1561
2.Sesame cake extracted with Acetone	20.0	590 118	1661 332	--	2251 450

3.Sesame cake extracted with Ethanol	14.0	569 80	2608 365	892 125	4089 570
4.Sesame cake extracted with Ethyl acetate	12.4	926 116	5730 720	2120 266	8776 1102
5.Sesame cake extracted with Isopropanol	1.5	67.8	394	22	484
6.Sesame cake extracted with Hexane(ie.cakeoil	4.9	---	3220 157	trace	3220 157
7.Sesame seed extract with Methanol	28.5	3830 488	3998 510	2057 262	9885 1261
8.Purified extract from cake as per our process	5.0	22676 1108	1,05738 5168	12,500 611	1,40,914 6887
9.Sesame seed extract purified as per our process.	7.2	16733 2351	3951 555	2233 314	22917 3220

* Conditions of extraction: Soxhlet extraction by respective solvents for 16 hours in taking 10 g sample under same conditions. The extract weight is expressed as % of raw material weight. The antioxidant (lignan) content is expressed as 'parts per million' ie. milligrams of lignans present in 1 kg. of the extract. Alternately, the concentartion of lignan as milligrams present in 1 Kg. of the raw material is also calculated and given in blue ink.

The results clearly show that by our process, there is enrichment of antioxidant compounds by 4.5 times at least on raw material weight basis itself.

The main advantages of this invention are:

- The development of a process for the extraction of natural antioxidants from sesame seed/cake effective in protecting vegetable oils, the antioxidant compounds being reported to have beneficial effects.
- The extract is capable of protecting vegetable oils with high unsaturation like soyabean oil at a lower concentration range containing 3 to 30 ppm levels.
- To utilise sesame cake for the extraction of natural antioxidants to be used to protect vegetable oils, thus resulting in value addition to cake.
- After extraction of antioxidants from cake, the cake meal still contains proteins, fibre and sugars and fat to be utilised as cattle feed.

All Annexures that are mentioned in this description (e.g., Annexures IV, VI, VII, and any other Annexures that are mentioned in the description) are incorporated herein by this reference.

All of the numerical and quantitative measurements set forth in this application (including in the examples and in the claims) are approximations.

The invention illustratively disclosed or claimed herein suitably may be practiced in the absence of any element which is not specifically disclosed or claimed herein. Thus, the invention may comprise, consist of, or consist essentially of the elements disclosed or claimed herein.

The following claims are entitled to the broadest possible scope consistent with this application. The claims shall not necessarily be limited to the preferred embodiments or to the embodiments shown in the examples.

We claim

1. A process for the extraction of natural antioxidants from oil seeds and by-products such as sesame seed / cake, the said process comprising defatting of the powdered oil seed or cake with hydrocarbon solvents at 25 to 85°C at a ratio of 1:1 to 1:7 for 3 to 24 hours, washing the defatted material with water or brine, at a ratio of 1:1-1:5, 3 to 8 times and drying the residue below 70°C for 6 to 10 hours, and extracting with organic solvents such as alcohols, esters, ketones, over a temperature range of 25 - 85°C for 10 hrs to 7 days and concentrating the said extract under reduced pressure of 150-100mm of Hg and dissolving the said concentrated extract containing 5 - 20% lignans in a permitted carrier such as pure ethanol/ethylene glycol/propylene glycol, stored under refrigeration till actual use.

2. A process as claimed in Claim 1 wherein the said defatting can be carried out by soaking the powdered seed/cake in hydrocarbon solvents such as pentane,hexane,heptane or mixtures thereof, in the above ratio for 1-5 hours' duration and removing the solvent and adding fresh solvent in the above ratio, at every interval and removing solvent.

3. A process as claimed in claim 2 wherein defatting can also be achieved by extracting the oil seed or cake in a soxhlet extractor with the above mentioned hydrocarbon solvents for 10-24 hrs.

4. A process as claimed in 2 and 3 wherein said defatted material can be water washed at 1:1 to 1:5 ratio, by stirring, 3-8 times at 1 hour interval.

5. A process as claimed in claim 4, wherein brine (3-10 % w/v sodium chloride solution) can be used at 1:1 -1:5 ratio for 1-3 washings,

followed by water washing at 1:1 to1:5 ratio subsequently for 1-4 times.

6. A process as claimed in claim 4/5, wherein the residue obtained is dried below 70 ^{0}C. by sundrying or by artificial means.

7. A process as claimed in claim 1/2 or 5/6 wherein the said meal after defatting/washing&drying is extracted with alcohols such as methanol, ethanol, isopropanol or ketones such as acetone, or esters such as ethyl acetate to get an antioxidant extract

8. A process as claimed in claim 7, wherein the said extract is concentrated preferably under vaccum(150-100mm Hg pressure).

9. A process as claimed in claim 8, wherein the said concentrate contains 5 to 20 % lignans, namely sesamol, sesamin, sesamolin, episesamin, lignan derivatives including glycosides, dimers etc. and lipids, sugars, proteins, minerals, browning(maillard reaction) products etc. is dissolved in ethanol or in any permitted food carrier and stored below 10°C.

10. A process as claimed in claim 9, wherein the antioxidant concentrate is capable of protecting commonly used vegetable oils like soyabean oil, safflower oil, sunflower oil, groundnut oil etc. against oxidative changes at concentrations ranging from 5 to 1000ppm and comparable with the protection offered by BHT at 200 ppm.

11. A process as claimed in Claim 10 by which the antioxidant extract can also be utilised for protecting foods, cosmetics, pharmaceuticals etc.

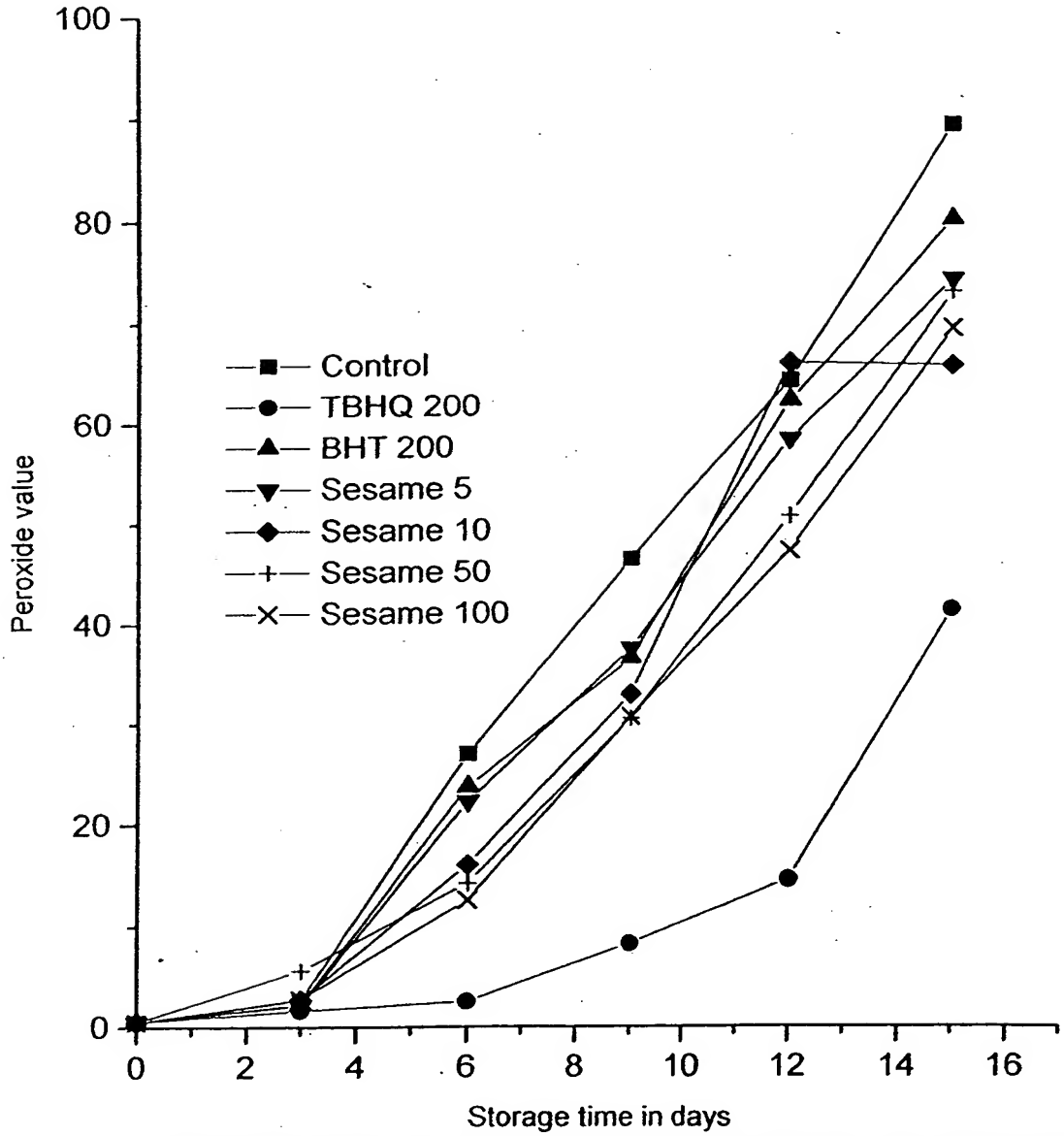

Fig.1:Peroxide value(m.equiv.O_2/kg) of soybean oil stored at 60^0C

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ANNEXURE VI

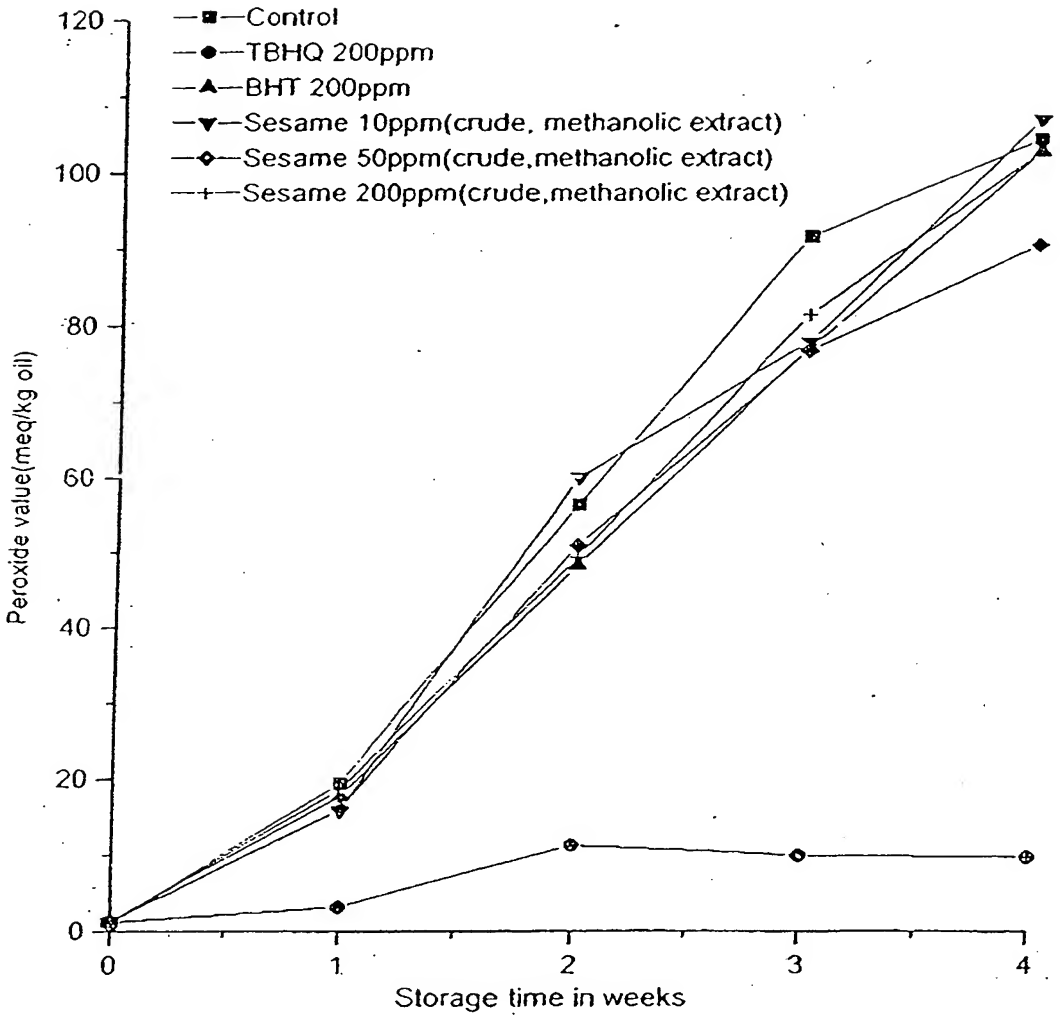

Fig.1a.Peroxide value(milliequiv. O_2/Kg) of Soybean oil stored at 60^{0}C

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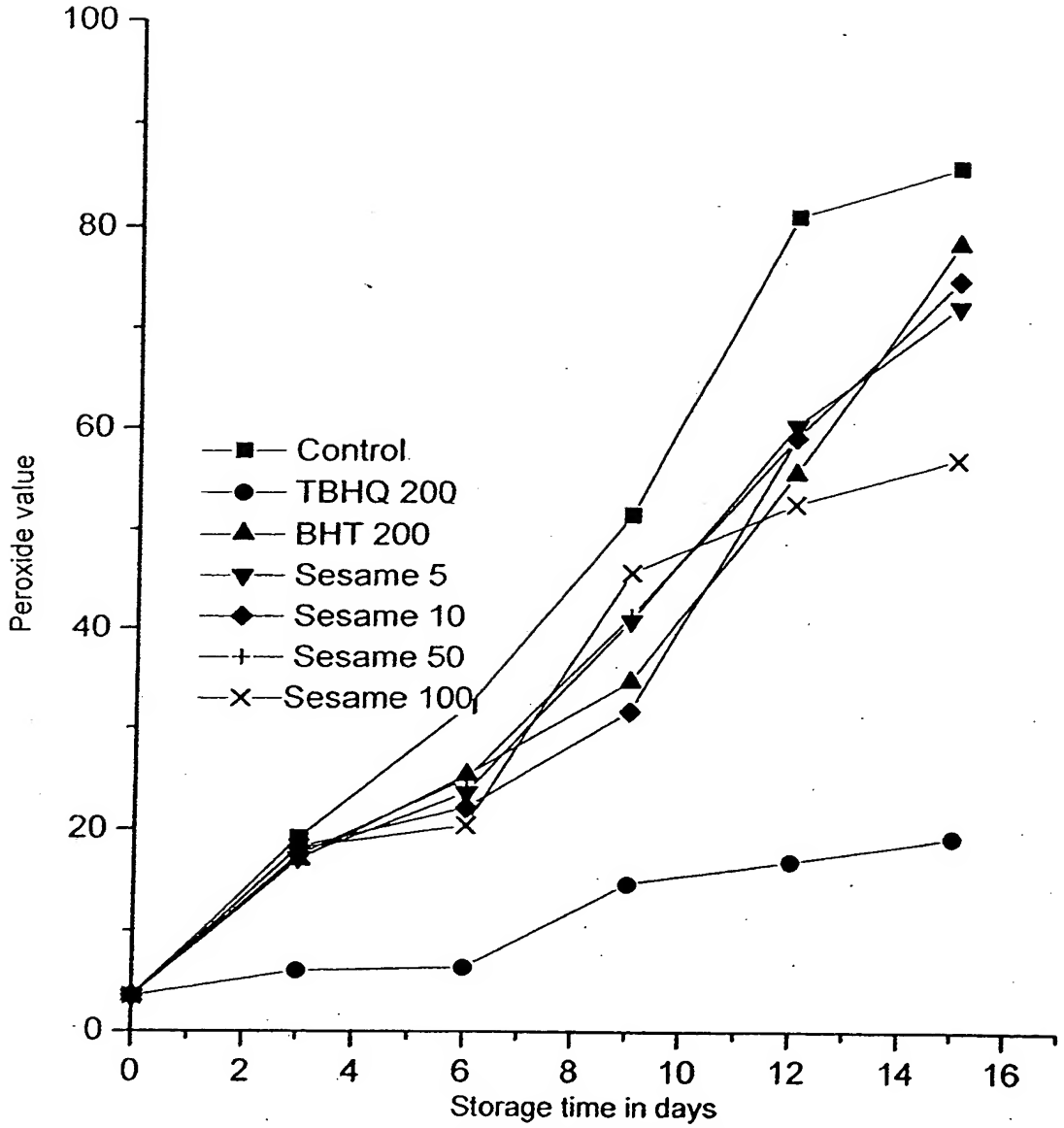

Fig.2.Peroxide value (m.equiv.O_2/Kg) of safflower oil stored at 60^0C

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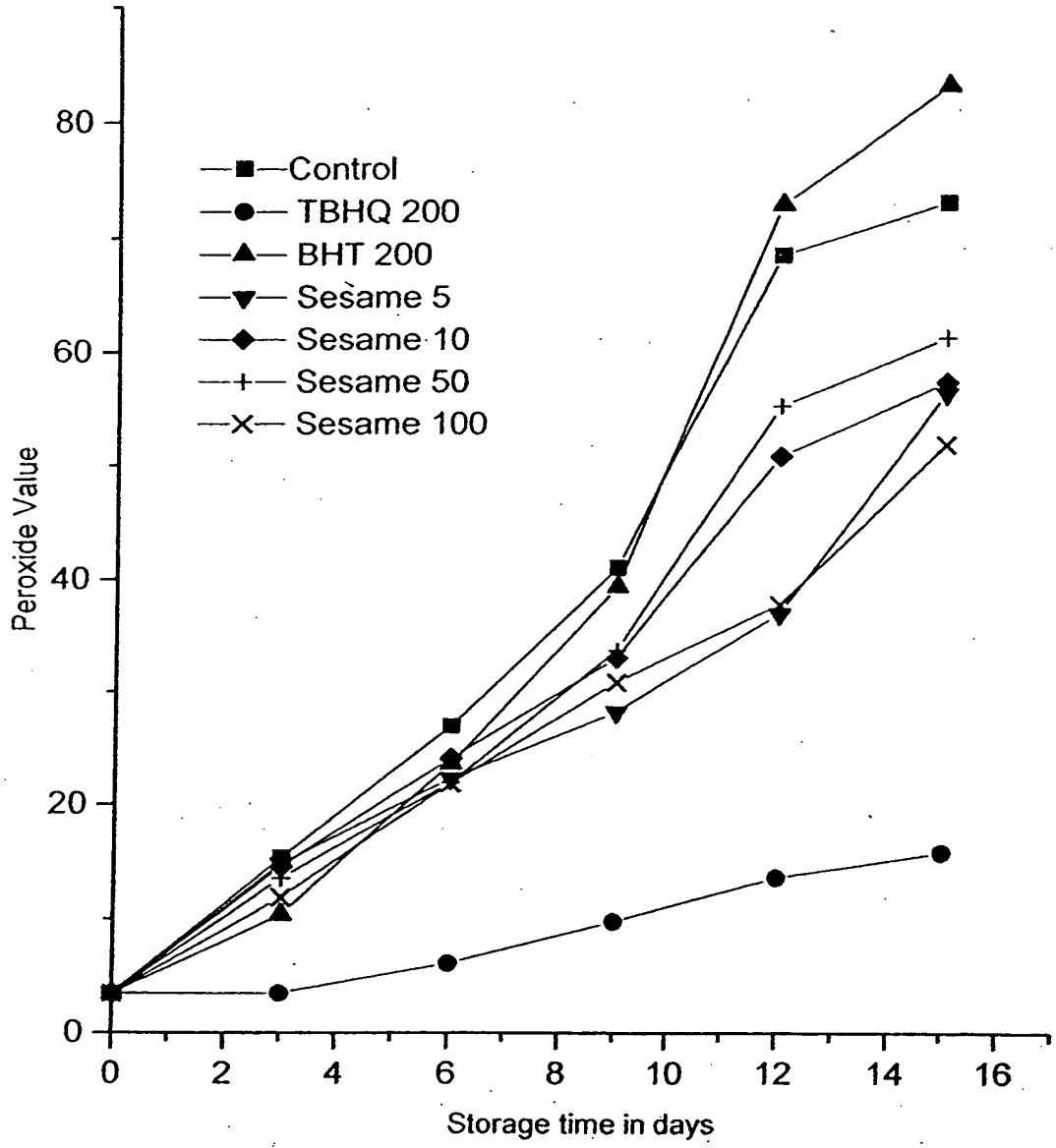

Fig.3.Peroxide Value(m.equiv.O_2/Kg) of Sunflower oil stored at 60 °C

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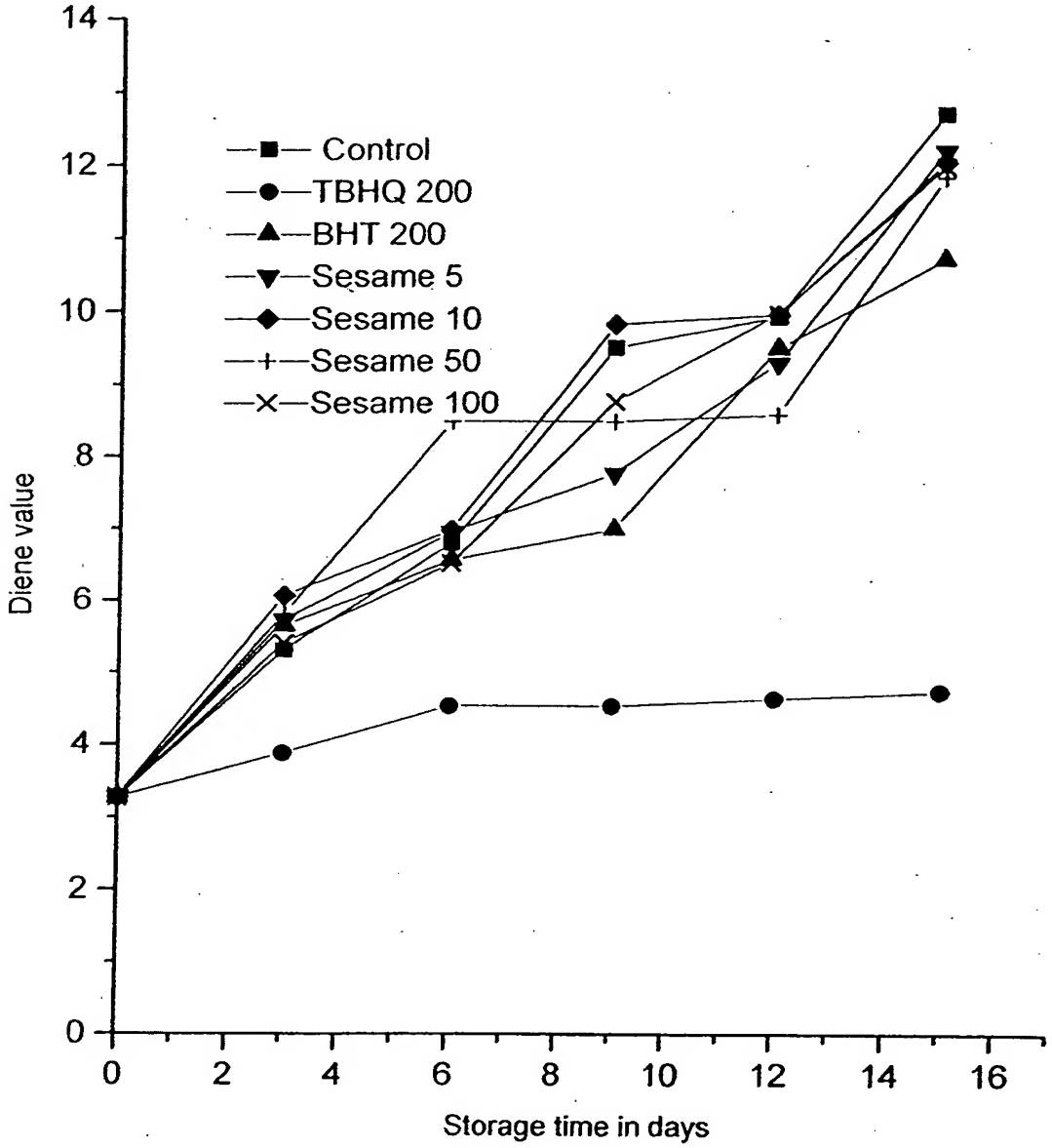

Fig.4.Diene Value of Safflower oil stored at 60 ° C

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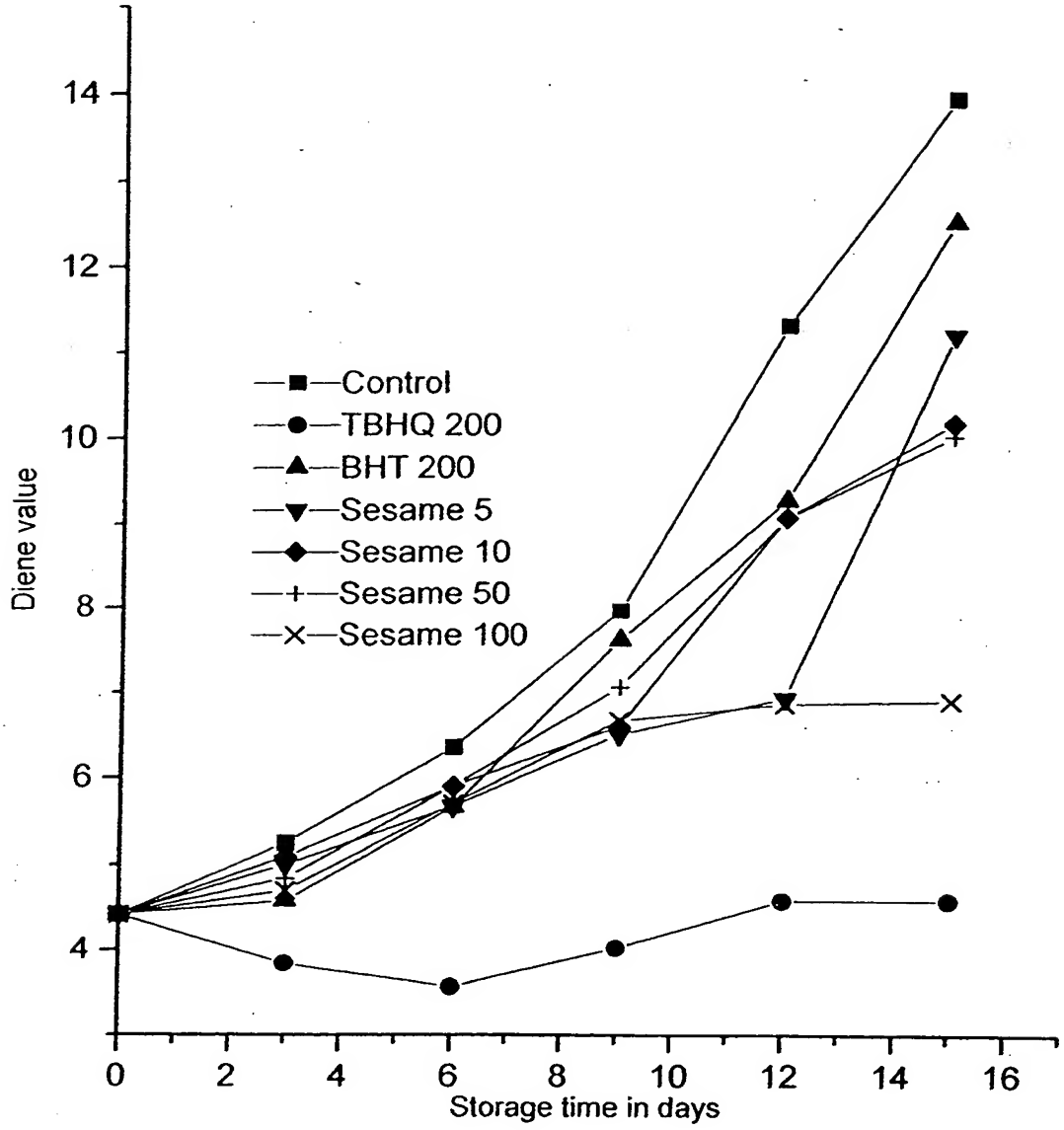

Fig.5.Diene Value of Sunflower oil stored at 60 ° C

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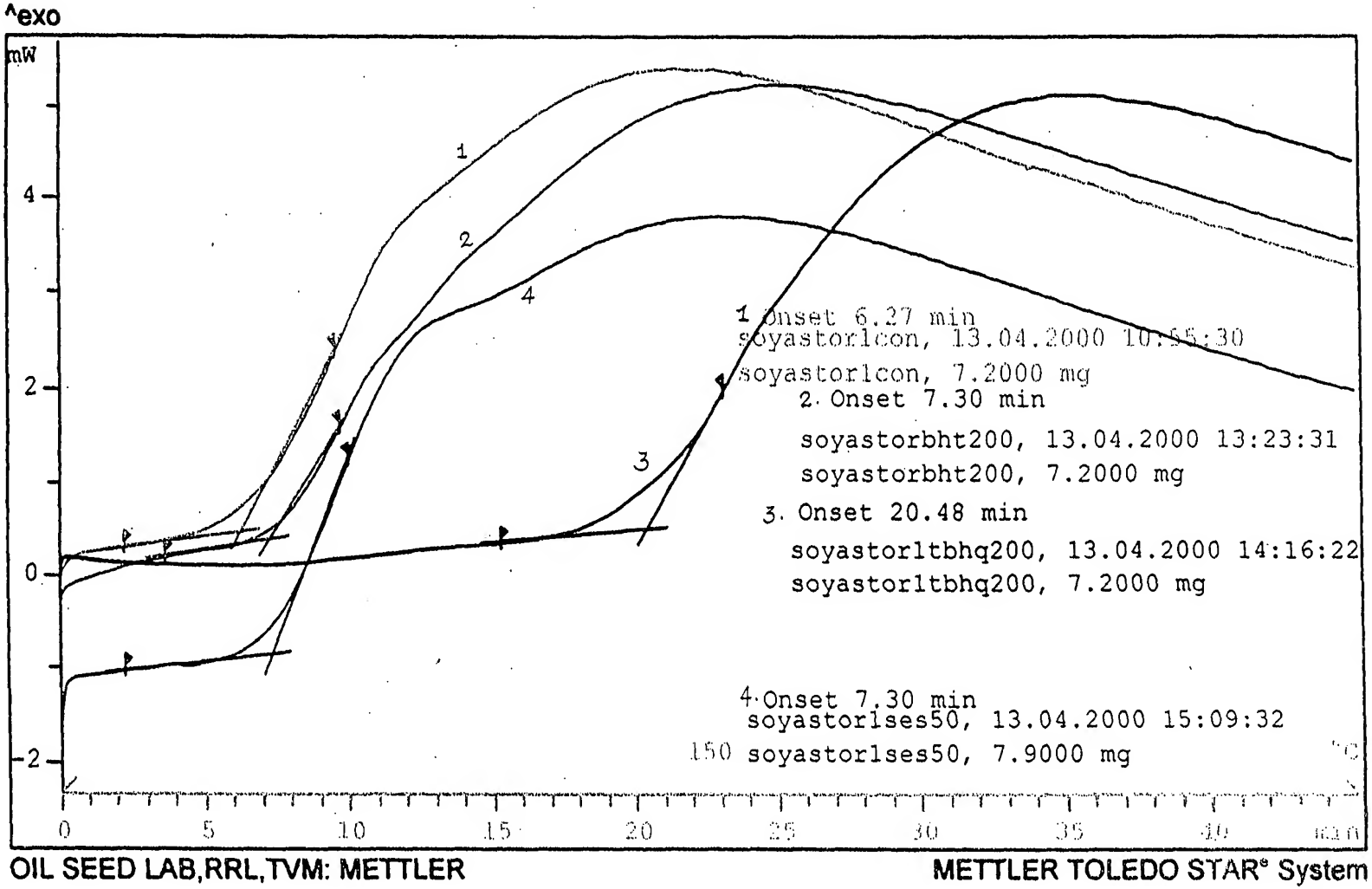

Fig.6. DSC profile of oxidative stability of soybean oil containing synthetic and sesame antioxidants at different concentrations (in ppm).

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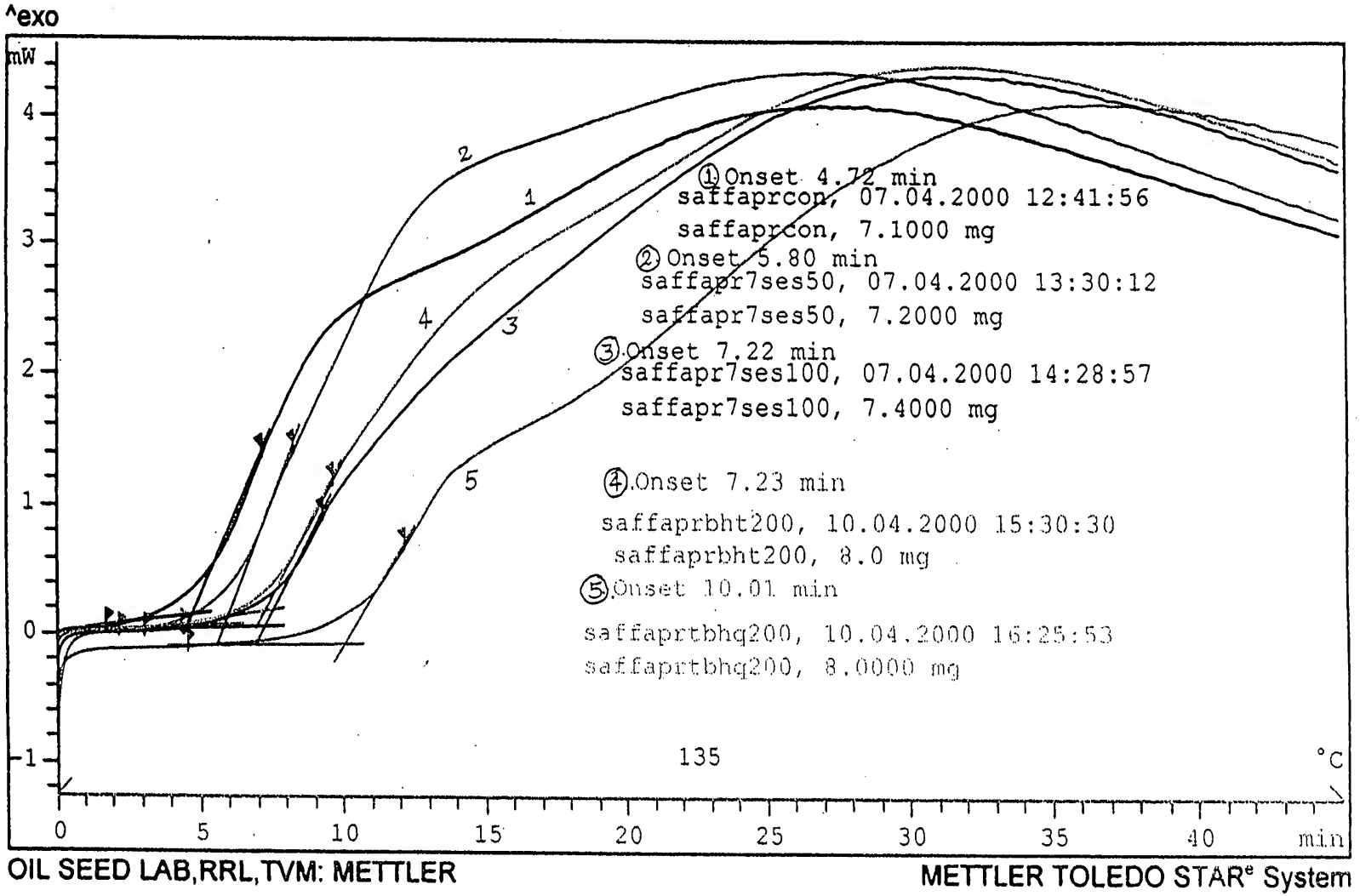

Fig.7. DSC profile of oxidative stability of safflower oil containing synthetic and sesame antioxidants at different concentrations (in ppm).

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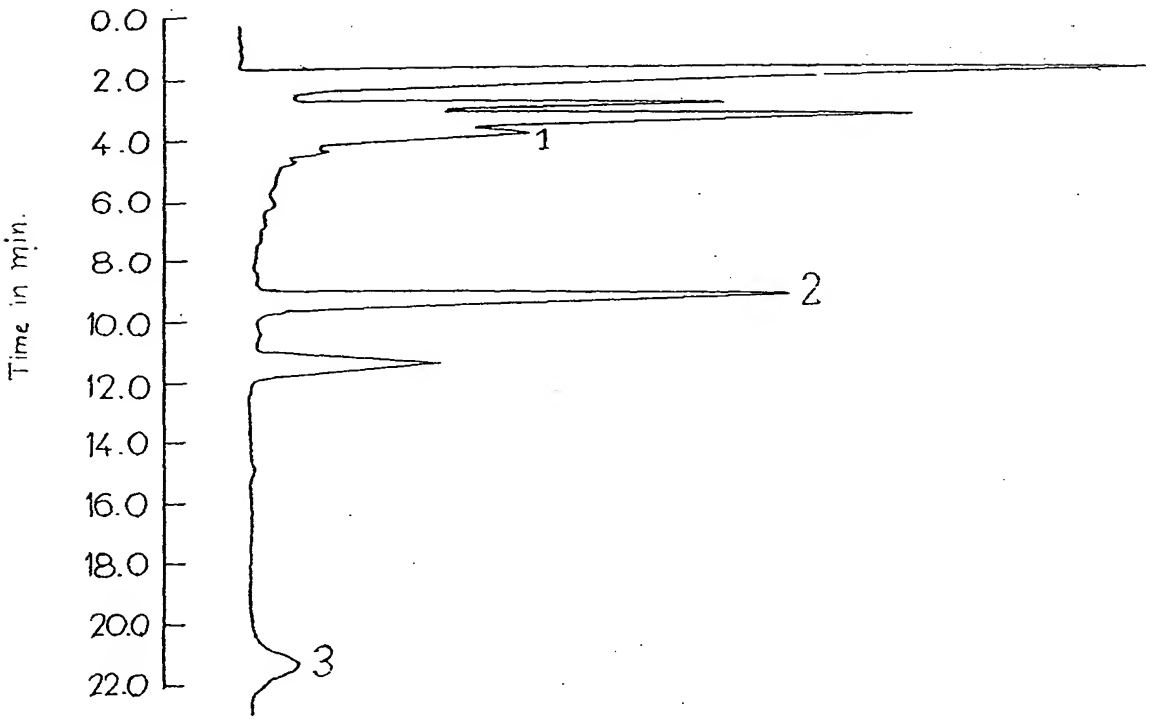

Fig.8: HPLC Profile of Sesame extract

(1) Sesamol (2) Sesamin (3) Sesamolin

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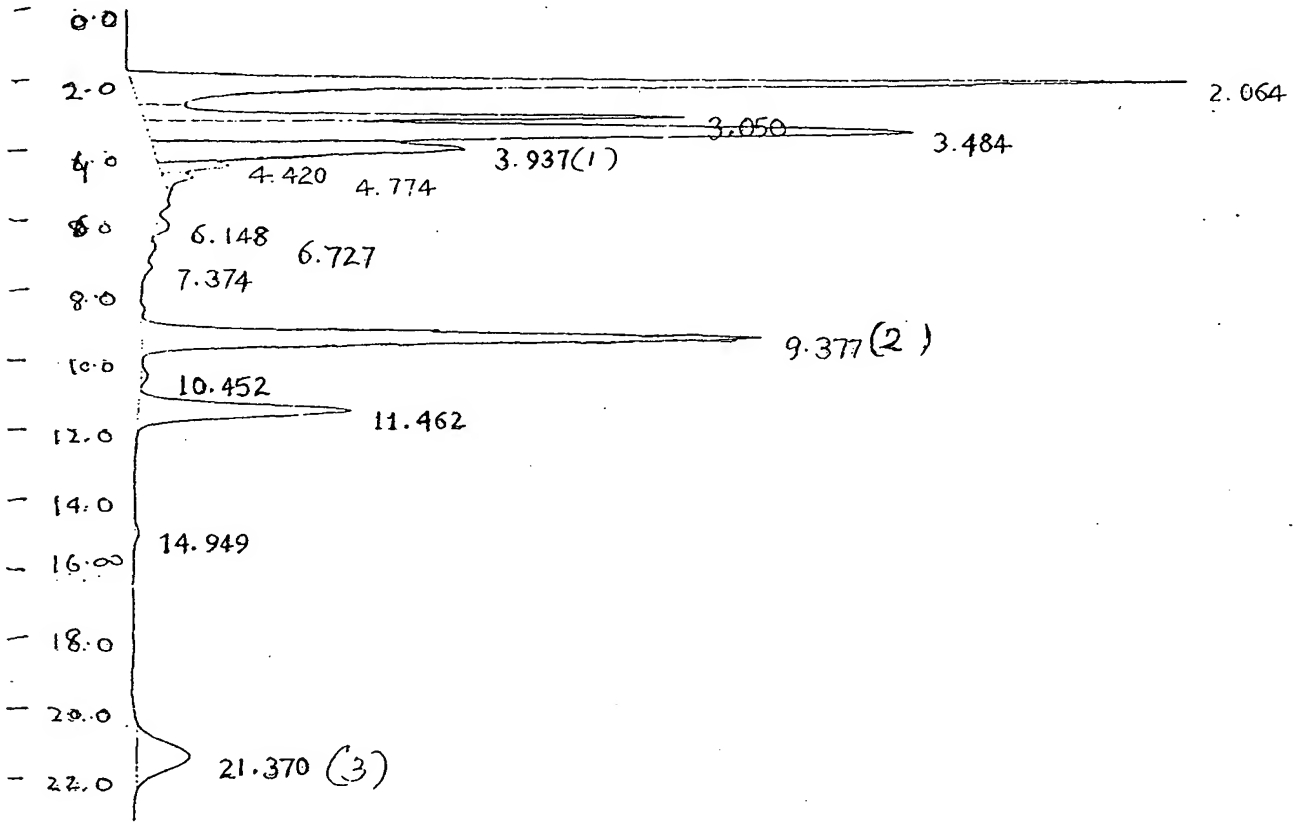
Fig(9)

SESAME CAKE EXTRACT - HPLC PROFILE (CRUDE EXTRACT)

7.7289g Cake → 1.5610g extract in 100ml methanol

C-R7A CHROMATOPAC CH=1 REPORT NO.=2 DATA=2:SESAME.C86 99 01 07 13:16:04



** CALCULATION REPORT **

Ext wt / Cake at basis

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
	1	2.064	271805	17739			24.6097	
glycoside	* 2	3.05	94763	8149	V		8.58	8471.60/1711.20
malonyl	* 3	3.484	274036	11539	V		24.8147	24495.22/4948.46
Sesamol	4	3.937	109023	4712	V		9.8711	2438.16/491.28 ppm
	5	4.42	15365	1055	V		1.3911	
	6	4.774	7501	449	V		0.6792	
	9	6.448	4446	166			0.3753	
	10	6.727	1393	73			0.1263	
	11	7.374	1040	73			0.0912	
Sesamin	13	9.377	199853	9343	V		18.095	4469.46/900.59
	14	10.452	1772	87			0.1604	
Sesamolin	15	11.462	81240	3217			7.3556	1816.88/366.08
	17	14.949	1019	52	V		0.0922	
Sesamolin	18	21.37	41508	779			3.7582	928.27/187.04
		TOTAL	1104465	57432			100	

Fig (10):

SESAME CAKE EXTRACT (PURIFIED) – HPLC PROFILE

10.230g Cake → 0.5g extract in 100ml MeOH

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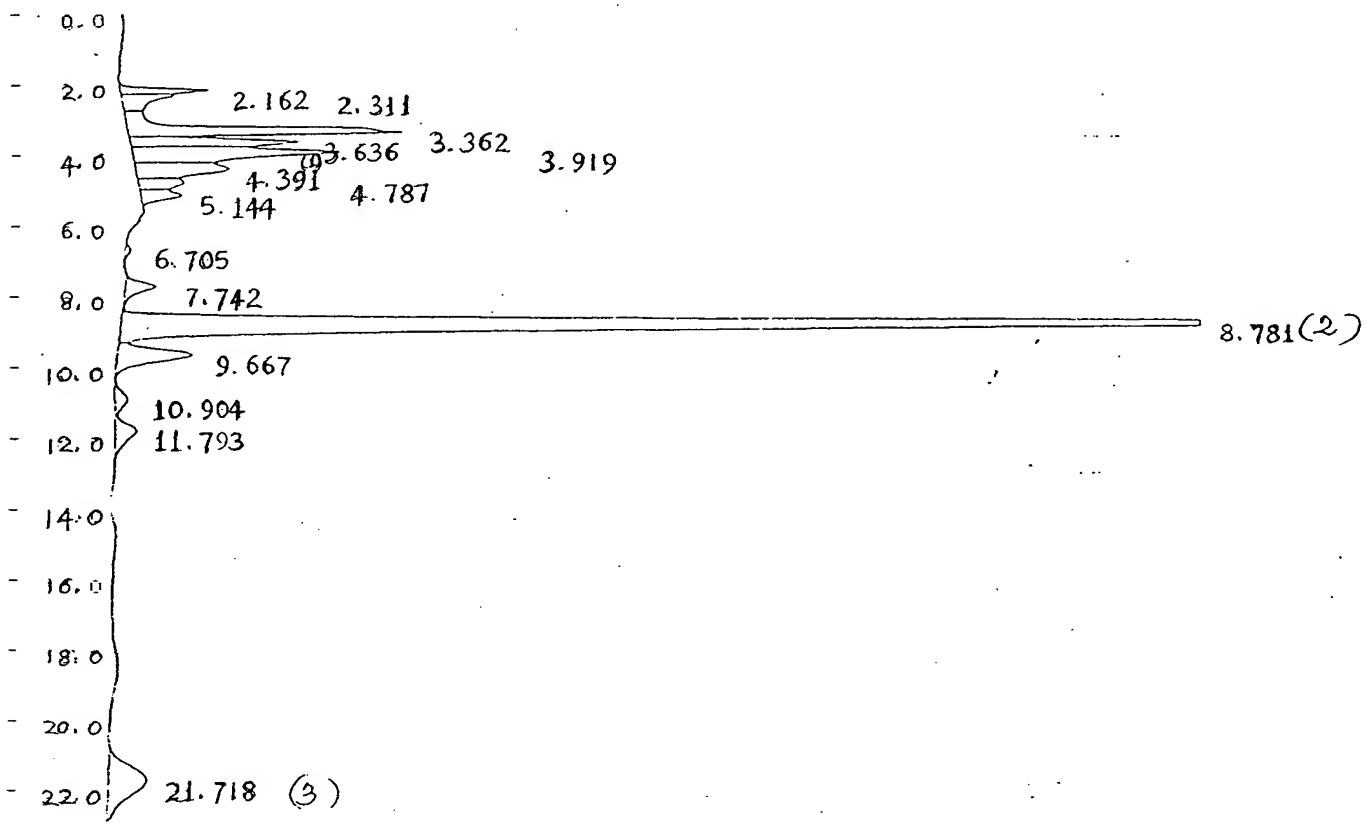


** CALCULATION REPORT **

CH	PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	1	2.162	11994	1299			1.7318	
	2	2.311	12797	767	V		1.8724	
	3	3.362	61832	4088	V		9.0466	
	4	3.636	33442	2503	V		4.8929	
	5	3.919	55292	5115	V		8.0898	
	6	4.391	29323	1391	V		4.2902	
	7	4.787	11002	666	V		1.6098	
	8	5.144	9328	599	V		1.394	
	9	6.705	1186	74			0.1736	
	10	7.742	8433	436			1.2338	
	11	8.781	373966	20037			54.7152	
	12	9.667	26034	1090	V		3.8091	
	14	10.901	5042	174	V		0.7377	
	15	11.793	10121	309	V		1.4809	
	16	21.718	33486	555			4.8993	
		TOTAL	683476	37124			100	

Ext. wt basis / Cake wt. basis

17257.31 / 843.46

9333.66 / 456.19

22675.75 / 1108.29

10573.43 / 516.800

4530.63 / 221.43

12500.04 / 610.94

Abstract

Process for extraction of Antioxidants from Sesame seed/cake.

An antioxidant extract from sesame seed / cake is prepared by employing selective extraction techniques and purification methods and that which can be effectively utilised as a substitute for synthetic antioxidants for the protection of vegetable oils, foods, cosmetic/pharmaceutical preparations etc.

Legend for abbreviations used in the patent text /Figures.

1. ppm : parts per million. Alternately this represents milligram of the chemical/extract per Kilogram of the oil (carrier/matrix).

2. BHT :Butylated Hydroxy Toluene(BHT). Synthetically produced antioxidant permitted in Foods/oils etc. but reported to be deleterious to health and likely to be banned in future. Maximum usage level is 200 ppm ie.(0.02%).

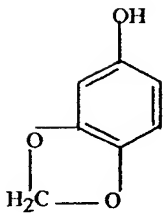
3. TBHQ :tert- Butyl Hydro Quinone.Synthetic antioxidant permitted for use in foods and oils/fats in few countries only. Maximum usage level is 200 ppm (ie. 0.02 %).

4 . PV : Peroxide Value, a measure of the oxidative damage of the unsaturated oils. It is expressed as 'milli equivalents of oxygen per Kilogram of oil/fat '. When oils and fats are protected by antioxidants against oxidative changes, the Peroxide Value (PV) of such samples would be lower than the control sample of oil without added antioxidants.

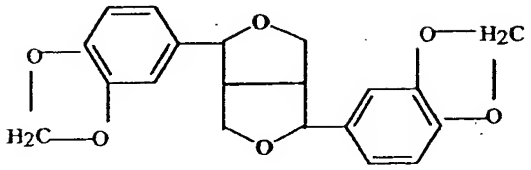
5. AV :Anisidine Value. This is a measure of secondary oxidation status of oil. Expressed as numerical value only.

6. DV :Diene Value. This is also a measure of primary oxidation status of the vegetable oil and is expressed as a numerical value only.

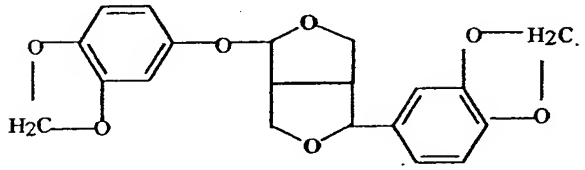
7. EC_{50} Antioxidant concentration needed to decrease the initial [DPPH·] concentration by 50%.
This value is inversely related to the measure of free radical quenching efficiency of the
antioxidant.



Structure 1.Sesamol



Structure 2.Sesamin



Structure 3.Sesamolin

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